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FERMENTATION OF PENTOSE SUGARS TO ETHANOL  
AND OTHER NEUTRAL PRODUCTS BY MICROORGANISMS

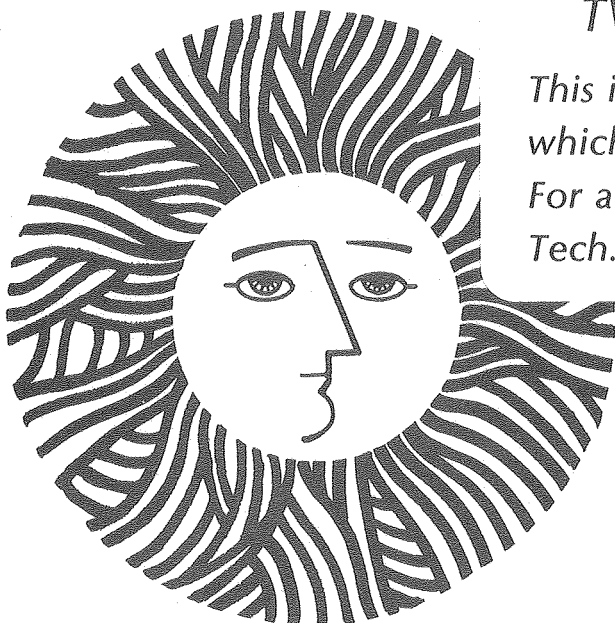
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FERMENTATION OF PENTOSE SUGARS  
TO ETHANOL AND OTHER NEUTRAL  
PRODUCTS BY MICROORGANISMS

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## SUMMARY

The current and projected shortage of petroleum and natural gas has led to renewed interest in processes for the microbial conversion of renewable biomass resources to liquid and gaseous fuels. Pentose sugars represent a significant fraction of the total fermentable carbohydrate content of biomass. A number of biochemical pathways are known for the conversion of pentose to ethanol and other neutral products. Beside ethanol, potential neutral fermentations products include 2,3-butanediol, acetone, isopropanol, butanol and hydrogen. Other products include carbon dioxide and organic acids. Specific ethanol-producing fermentations are reviewed, and future directions for research and development are suggested.

## INTRODUCTION

Due to concern about the cost and availability of petroleum and natural gas, interest is increasing in processes which make use of renewable resources, such as plant biomass, for the generation of liquid fuels and chemical feedstocks.

The major organic components of land-plant biomass are cellulose, hemicellulose and lignin. Cellulose is a high molecular weight, insoluble, linear  $\beta$ , 1-4 linked polymer of D-glucose. Lignin is a high molecular weight insoluble, three-dimensional random polymer of sinapyl, coniferyl and p-coumeryl alcohols. The hemicelluloses are alkali-soluble, linear and branched heteropolymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid. In addition, homopolymers of xylose, mannose and galactose are known to occur. Individual sugars may be methylated and acetylated. Whistler and Richards (1) have reviewed the chemistry of the hemicelluloses.

The energy content of biomass can be utilized most simply by direct burning. It is also possible to convert biomass to higher value gaseous and liquid fuels and chemicals by thermochemical and biological means (2). Thermochemical processes usually produce complex mixtures of products and require high temperatures and pressures. In contrast, fermentative bio-conversion processes operate at lower temperatures (ca. 25-60°C) and at atmospheric pressure and produce only a very few major products.

While a number of modern processes have been designed or proposed for the conversion of cellulose, starch, and sugar to fuels and chemicals (2-4), less interest has been shown in bioconversion schemes involving pentose sugars, although as can be seen in Table 1, these can represent a third or more of the total carbohydrate content of biomass (5).

It is my intention in this article to review the methods currently available for biologically converting pentose sugars, which are the major constituents of hemicellulose, to ethanol and other natural products. These products can be used as clean-burning liquid fuels, solvents or chemical feedstocks. Pentose fermentations which do not yield ethanol as a significant product (> 5% yield, gm/gm substrate fermented) will not be considered.

#### EARLY STEPS IN THE METABOLISM OF PENTOSE AND PENTITOLS

The chemical structures of the eight aldopentoses and four pentitols are shown in Figure 1. Of this group only two of the pentitols (ribitol and D-arabitol) and three of the aldopentoses (D-ribose, L-arabinose and D-xylose) are found in significant quantities in nature (6). The others have been termed "unnatural" carbohydrates by Mortlock (6) due to their rarity in or apparent absence from the natural environment. The abundant species are indicated by underlining in Figure 1.

In most micro-organisms the early steps in pentose and pentitol metabolism involve conversion of the starting material to the common intermediate D-xylulose-5-phosphate. The organism most studied with respect to the early steps in pentose and pentitol metabolism is the bacterium Aerobacter aerogenes (Klebsiella pneumoniae), strain PRL-R3. The wild-type of this organism is able to utilize all five of the common pentoses and pentitols as sole sources of carbon and energy. In addition mutants have been selected which can completely metabolize all the "unnatural" forms as well (7). Figure 2 shows the enzymatic steps involved in the conversion of eleven of these twelve compounds to D-xylulose-5-phosphate (8). L-ribose was not available for testing as a potential substrate (6).

The "new" enzyme activities expressed by the mutants were actually already present, their functions being to process structurally related natural sugars. The actual mutational steps involved loss of repressive regulation or altered inducer specificity for the pre-existing genes. The specific events are discussed by Mortlock (6). Evolutionary strategies used by micro-organisms to construct new metabolic pathways are reviewed in more general terms by Hegeman and Rosenberg (9) and by Clarke (10).

With the exception of D-ribose it can be seen that this organism processes these substrates in a stereotyped manner. Pentitols are dehydrogenated to their respective 2-oxo derivatives while pentoses are isomerized. All the pentuloses formed participate in existing pathways for metabolism of the common pentoses and pentitols (L-xylulose is formed from naturally occurring L-ascorbic acid). For those pentoses studied the same metabolic approach is used by strains of Escherichia coli (6) and a number of other groups of pentose-fermenting bacteria (11).

Among the yeasts a different pattern of stereotyped reactions appears to be common for the early steps in the metabolism of pentoses. Pentoses are first reduced to the corresponding pentitol by pyridine nucleotide-linked dehydrogenases and then re-oxidized by a second set of dehydrogenases to the corresponding pentulose. This pattern of metabolism has been described by Barnett (12) and is shown in Figure 3. It may be common among the fungi in general (13).

A third mechanism of pentose utilization is illustrated by the D-ribose pathway (Figs. 2 and 3). This sugar may be phosphorylated first and then isomerized to the pentulose. D-arabinose is metabolized by some strains of Propionibacterium pentosaceum in this manner (14), but not to ethanol. As more organisms are studied, other permutations of these basic reactions may be found.

#### CONVERSION OF PENT(UL)OSE PHOSPHATE TO PYRUVIC ACID

All pentose-fermenting, ethanol-producing bacteria so far studied appear to use a combination of the pentose-phosphate and Embden-Meyerhoff pathways for the conversion of pent(ul)ose phosphate to the key intermediate pyruvic acid. The combined pathways are shown in Figure 4. The result of these enzymatic operations is the conversion of three moles of pen(ul)ose phosphate to five moles of pyruvic acid with a net synthesis of five moles of ATP and five moles of reduced pyridine nucleotide. If the substrate is a pentitol, and extra mole of reduced pyridine nucleotide will be produced per mole of substrate fermented.

Among the fungi, only members of the genus Fusarium have been reported to produce significant quantities of ethanol from pentose (15). In resting cell suspensions D-xylose is fermented to approximately equimolar amounts of ethanol,

CO<sub>2</sub> and acetic acid (16)

Carbon-14 labeling experiments have demonstrated that in this organism D-xylulose-5-phosphate is cleaved between carbon atoms 2 and 3 rather than being metabolized by the pentose-phosphate-Embden-Meyerhoff pathway. The product labeling pattern indicates that acetic acid is derived from the 2-carbon fragment, and ethanol and CO<sub>2</sub> are derived from the 3-carbon fragment (16). A similar pattern is shown by the lactic acid bacteria. Acetate is formed from the 2-carbon fragment, but the 3-carbon fragment is reduced to lactate. The enzyme responsible for the C-2, C-3 cleavage is phosphoketolase (17). The steps in the metabolism of xylulose-5-phosphate to acetic acid and pyruvate are shown in Figure 5.

#### CONVERSION OF PYRUVIC ACID TO ETHANOL AND OTHER FERMENTATION PRODUCTS

A relatively small number of metabolic pathways is responsible for the conversion of pyruvic acid to ethanol and other commonly encountered fermentation products. Furthermore, not all of these pathways are present in a given organism so that the number and amounts of products formed are often characteristic of a genus or species. Although a large number of microbial species is capable of producing ethanol by the fermentation of hexoses and pentoses, only a few have been found which produce it in significant quantities.

##### A. Production of Ethanol by *Fusarium oxysporum* and Yeasts

Figure 6 shows the steps used by *F. oxysporum* and yeasts for the conversion of pyruvic acid to ethanol and CO<sub>2</sub>. Reduced pyridine nucleotide used for the reduction of acetaldehyde is generated by the oxidation of glyceraldehyde-3-phosphate to 1,3-biphosphoglyceric acid (Fig. 4).



As mentioned earlier, a significant quantity of acetic acid, as well as ethanol, is produced from xylose by resting cells of Fusarium oxysporum. However, growing cultures of this mold produce higher levels of ethanol and CO<sub>2</sub> and much less acetate. Table 2 shows data from such a fermentation (18). The CO<sub>2</sub> yield is probably understated due to losses while sampling.

The difference between the ethanol yields in resting and growing cells suggests that either growing cells use a different metabolic scheme for pentose fermentation or that they are able to reduce acetate to ethanol.

Many yeasts are able to metabolize pentoses and pentitols aerobically, yet of over 400 species studied by Barnett (12) none was able to ferment these substrates. Nevertheless, cell-free extracts of some strains of Brewers yeast have been shown to be able to ferment D-ribose (19) and D-ribose-5-phosphate (20) to ethanol and CO<sub>2</sub>. The enzymatic studies of Sable (21) and the <sup>14</sup>C-label experiments of Gibbs, et al. (19) suggest that the fermentation is carried out via the pentose-phosphate-Emden-Meyerhoff pathway.

Both Dickens (20) and Gibbs, et al. (19) noted that pentose fermentation was incomplete. For every mole of ribose or ribose-5-phosphate that disappeared only approximately one mole each of ethanol and CO<sub>2</sub> was produced. Gibbs, et al. suggested that the missing carbon had accumulated in the pool of metabolic intermediates. When glucose was fermented, one mole of glucose was stoichiometrically converted to 2 moles of ethanol and 2 moles of CO<sub>2</sub> (20).

The above work suggests that at least some yeast strains have the enzymatic potential to ferment pentose sugars to equimolar amounts of ethanol and CO<sub>2</sub>. Presumably, whole cells do not ferment these substrates because of impermeability and/or unfavorable patterns of enzyme regulation.

## B. Fermentations Carried Out by Bacteria

### 1. The Mixed Acid and Mixed Acid + 2,3-Butanediol Fermentations.

The major products of the mixed acid fermentation are ethanol,  $\text{CO}_2$ , and acetic, succinic, lactic and formic acids. Formic acid may be replaced by an equivalent amount of  $\text{H}_2$  and  $\text{CO}_2$ . In the butanediol fermentation these same products may be present, and in addition 2,3-butanediol is produced. Figure 7 shows the products which are typical of the mixed acid and butanediol fermentations and the biochemical steps in their formation.

The mixed acid-butanediol fermentation is characteristic of (but not limited to) members of an assemblage of facultatively anaerobic bacteria classified as the enteric group (22). Table 3 lists by genus the members of this group and indicates the type of fermentation carried out. The formic hydrogenlyase enzyme complex is responsible for the conversion of formic acid to hydrogen and carbon dioxide. In its absence formic acid accumulates. With the exception of the genus Photobacterium at least some of the members of each genus are able to ferment one or more of the pentoses and/or pentitols (23).

The relative amounts of products formed in any fermentation will depend on the organism, the substrate and the environmental parameters chosen. This is illustrated in Table 4 for the mixed acid fermentation conducted by Escherichia coli. Acidic conditions favor the production of  $\text{H}_2$  and  $\text{CO}_2$  at the expense of formic acid. Glycerol and butyric acid are not normally found in mixed acid fermentations but are produced by this strain.

In addition to the enteric organisms listed in Table 3, five other organisms should be noted which conduct a mixed acid fermentation and which have been described as fermenting five-carbon sugars. Three also ferment cellulose.

Spirochaeta stenostrepta, strain Z1, isolated by Canale-Parola, et. al  
(66) ferments L-arabinose, D-ribose and D-xylose in addition to several  
hexose mono- and disaccharides (67). Fermentation of glucose by growing cells  
yielded (per 100 m moles of sugar fermented): ethanol, 84 m moles; acetic  
acid, 93 m moles; lactic acid, 10 m moles; CO<sub>2</sub>, 140 m moles and H<sub>2</sub>, 180 m moles  
(66). Thirteen percent of the substrate carbon was unaccounted for. Higher  
yields of ethanol from glucose were produced by resting cell suspensions (67).  
Fermentation balances using pentoses as substrates have not been reported.

Ruminococcus albus, a strict anaerobe described by Bryant, et al. (24), ferments cellulose, cellobiose and xylan, a homopolymer of D-xylose. Major products of cellobiose fermentation are  $H_2$ ,  $CO_2$ , acetic acid, ethanol, formic acid and some succinic and lactic acid. D-xylose and L-arabinose were not fermented except by one strain. It was suggested that xylan fermentation might proceed after conversion to xylobiose by a process analogous to cellulose fermentation which often occurs via cellobiose rather than glucose (24).

In his original description of R. albus, Humgate (25) noted one strain (56-2) which fermented L-arabinose well. A fermentation balance for this organism growing with cellulose is shown in Table 5. Fermentation balances for pentose sugars have not been reported.

Clostridium thermocellum and C. thermocellulaseum, two other cellulolytic anaerobes, also conduct a mixed acid fermentation of cellulose. C. thermocellulaseum was reported by Enebo (26) to ferment cellulose to ethanol, formic, acetic, lactic and succinic acids, carbon dioxide and hydrogen. Both arabinose and xylose were fermented, but no product balances were reported for these substrates.

One strain of C. thermocellum examined by McBee (27) fermented cellulose to the products shown in Table 5. L-arabinose, but not D-xylose, was also fermented, but fermentation balances were not reported. Other strains of C. thermocellum studied by the author produced the same products listed in Table 5 plus others which were not identified. Formic acid is not produced by C. thermocellum strains currently being studied (J.G. Zeikus, personal communication).

Quite recently fermentation balances have become available for the

thermophilic anaerobe Clostridium thermohydrosulfuricum (68). This organism is able to ferment glucose to ethanol and  $\text{CO}_2$  almost quantitatively (1.8 mole of each product per mole of glucose fermented). Xylose is also fermented, but product yields on this substrate have not yet been reported.

As mentioned earlier, in the butanediol fermentation some or all of the products of the mixed acid fermentation may be formed, and in addition 2,3-butanediol is synthesized. Butanediol exists in three stereoisomeric forms: D(-), L(+), and meso, and different organisms yield one or more of these isomers in fermentations (28). The chemical reactions and chemical and

physical properties of 2,3-butanediol are discussed in an extensive review of the butanediol fermentation by Ledingham and Neish (28). Long and Patrick have reviewed the process more recently (29).

In addition to the members of the enteric group listed in Table 3, members of the genus Bacillus and Clostridium also produce ethanol and 2,3-butanediol in varying amounts (23,30). Strains of four species of bacteria have been isolated from nature which have demonstrated the ability to produce major amounts of ethanol and 2,3-butanediol and minor amounts of organic acids from pentose and hexose sugars. Comparative data are shown in Table 6. The pH of the Aeromonas, Bacillus and Aerobacter indologenes fermentations was controlled to a final value of 5.6-5.8 (31) by the presence of excess  $\text{CaCO}_3$ . Starting pH's of  $\text{CaCO}_3$ -controlled fermentations tend to be above eight, however. The pH of the Enterobacter aerogenes fermentations was controlled rigorously from the outset by automated equipment. A low pH favors the production of butanediol and ethanol, while a high pH favors the formation of organic acids. This is due in part to the fact that the first enzyme in the butanediol pathway,  $\alpha$ -acetolactate synthase, is formed and active under slightly acidic conditions directing the flow of pyruvate toward the formation of 2,3-butanediol (32).

In addition to the data shown, quantitative assessments have also been made by Neish and Simpson (33) and by Altermatt, et al. (34) of butanediol fermentations of pentose sugars carried out by A. aerogenes. In these experiments, however, alkaline conditions were maintained throughout the fermentations, and butanediol levels were, consequently, low.

Much of the work on the butanediol fermentation was undertaken during World War II since it was known that 2,3-butanediol could be chemically

converted to 1,3-butadiene, the major monomeric constituent of buna-S-rubber, as well as to a number of other useful chemicals (28). With the end of the war and the rise of a chemical industry built on cheap petroleum and natural gas, fermentative processes for the production of butanediol (and a number of other chemicals) fell into disuse.

A major expense in the butanediol fermentation is recovery of the product from a rather dilute aqueous solution by distillation. Butanediol has a boiling point at atmospheric pressure of approximately 180°C. Processes for the production of butanediol and ethanol from pentose-containing biomass and biomass wastes have been described by Murphy and Stranks (39) and Perlman (40).

## 2. The Acetone-Butanol-Ethanol Fermentation

The acetone-butanol-ethanol fermentation was discovered by Pasteur in the mid 19th Century. It became commercially interesting in the early 1900's because butadiene could be made from butanol. Expanded production of plantation rubber, however, caused the fermentation-based butadiene-synthetic rubber industry to decline. With the outbreak of the First World War demand for acetone increased dramatically, and new fermentation plants were built. Acetone was used as a solvent in the manufacture of the explosive cordite and in the preparation of aircraft "dopes". There was little demand for butanol by the allies during this period, although it was the basis of the synthetic rubber industry in Germany. At the end of the war, acetone demand fell, however, it was found that butyl acetate, derived from butanol, was an excellent solvent for nitro cellulose laquers which were being used in the growing automobile industry. The acetone-butanol fermentation continued as a significant industrial process until the end of the Second World War when it was displaced by cheaper competitors derived from petroleum and natural gas.

One plant continues to operate in South Africa and is described by Spivey (41). A more detailed history of this fermentation is presented by Prescott and Dunn (30) and by McCutchan and Hickey (42).

Although the amount of ethanol produced in the acetone-butanol fermentation is small, a discussion of this process is included because at one time it was commercially successful, and the products are useful as solvents, chemical feedstocks or fuels.

Figure 8 shows the biochemical steps involved in the conversion of pyruvate to butyric acid, butanol, acetone and isopropanol. Ethanol is produced by the reduction of acetyl CoA as shown in Figure 7. It should be noted that in the Clostridia, which conduct this fermentation, the conversion of pyruvate to acetyl CoA,  $\text{CO}_2$  and  $\text{H}_2$  does not involve formation of formic acid as an intermediate. Table 7 shows fermentation yields for several Clostridium species grown with glucose as a substrate. Total solvent yields for C. acetobutylicum using D-xylose as a substrate are similar (44).

In a typical industrial fermentation using C. acetobutylicum the yield of volatile products (acetone + butanol + ethanol) is 30-35% based on the grams of products formed per gram of sugar fermented. Butanol, acetone and ethanol are produced in a molar ratio of approximately 6:3:1 (30).

In a batch fermentation the concentrations of acetic and butyric acids rise initially, and the pH falls. At a pH of about 4.0, acid concentration begins to fall, and the amounts of butanol, acetone and ethanol increase as the pH rises (45). These observations suggested that the acidic fermentation products originally formed were being re-metabolized and converted to more reduced neutral products. This view was supported by the work of Speakman (46), who showed that addition of butyric acid to a fermentation increased the yield of butanol and by the work of Johnson, et al., (47), who



showed that addition of acetate increased the yield of acetone. The theory was confirmed by the work of Wood, et al. (48), who added  $^{13}\text{C}$ -labeled acids to fermentations and demonstrated incorporation of the label into neutral products. Interestingly, while 85% of the added butyric acid label was recovered in the butanol fraction (the balance was found in ethanol, isopropanol and acetic and butyric acids), only 15-19% of the added acetic acid label was found in acetone and isopropanol. Fifty percent of the label was found in the butanol fraction.

Wild-type C. acetobutylicum cells will not tolerate a butanol concentration much in excess of 1.3% (w/v) (49). This has limited the substrate concentration in industrial fermentations to about 6% (w/v) based on the product yields described earlier.

Acetone-butanol-ethanol fermentations have been conducted using the following biomass sources as substrates: xylose saccharification liquors from corncobs (50), cornmeal (51) acid-hydrolyzed oat hulls (52), waste sulfite liquor (53), and sugar derived from the acid hydrolysis of wood (54).

As with 2,3-butanediol, a significant fraction of the production cost of butanol can be attributed to its recovery from a dilute aqueous solution. The cheapest method for recovery at the present time is distillation. Important contributions toward making the process economical could be made by the production of higher solvent concentrations and by a cheaper process for separating butanol from the fermentation broth.

### 3. The Acetone-Ethanol Fermentation

Bacillus macerans, also once called Bacillus acetoethylicum, conducts a modified mixed acid fermentation of hexose and pentose sugars with the major liquid products being ethanol, acetic acid and acetone. Small amounts of

formic and lactic acids may also be produced. Gaseous products are carbon dioxide and hydrogen. Yields of acetone and ethanol obtained by fermentation of a number of carbohydrates are shown in Table 8 (55). These values are from un-optimized batch fermentations. Pathways for the production of the end products are shown in Figures 7 and 8.

As with the acetone-butanol fermentation, the yield of fermentation products depends on the pH at which the fermentation is conducted. Acidic conditions favor the production of neutral products at the expense of acids, while alkaline conditions have the opposite affect. The effect of pH on product yields is illustrated in Figure 9 (56).

As mentioned earlier, acetic acid (via acetyl co-enzyme A) is a precursor of acetone in the acetone-butanol fermentation of Clostridium acetobutylicum, and added acetate can be converted to acetone and butanol by this organism.

Arzberger, et al. (56) suggested that acetic acid may have a similar fate in fermentations conducted by B. macerans. They noted that no acetone could be detected in a culture until late in the fermentation and that the appearance of acetone was coincident with a drop in acid concentration. This suggestion is supported by the work of Speakman (57) who noted that during the fermentation of maltose the ratio of alcohol to acetone was high (>5.5) in the second day of the fermentation and gradually fell to a constant level of 2.2 by the eighth day. This indicated that acetone and ethanol could be formed independently.

de Mey and Wilssens (58,59) have conducted experiments which show that added <sup>14</sup>C-labeled acetate is, indeed, converted to acetone. However, in their experiments the largest part of the added label was found in the ethanol fraction. Whether this will be found to be generally true or whether the results were a function of the pH used (6.8) remains to be seen.

In older studies (60,61), it was reported that the pH optimum for growth of B. macerans was between 8.0 and 9.0 and probably closer to 8.0 (61). In these experiments culture pH's were not well-controlled. Our own preliminary studies using batch cultures with controlled pH (S. Rosenberg, unpublished data) indicate that the optimum pH for growth is approximately 6.0.

The ethanol-acetone fermentation has been conducted using the following acid-hydrolyzed or extracted biomass sources: corncobs (62), rice straw (63), oat and peanut hulls (64), and various grasses, straws and husks (61). In most of the cases listed above, the material being fermented is the hydrolyzed hemi-cellulose (pentose-rich) fraction of the biomass.

The maximum concentration of sugar fermented by wild-type B. macerans strains is about 3% (w/v) (60), but mutants have been selected which tolerate 7% sugar (63). Combined ethanol and acetone yields declined by about half in these fermentations, however.

One property of B. macerans which was noted in some of the earliest studies is its ability to produce a slime layer which allows the cells to stick to surfaces (60). Adding an inert material to the fermenter to increase the internal surface area allows a large inoculum to be retained facilitating semi-continuous operations.

#### DIRECTIONS FOR FUTURE RESEARCH AND DEVELOPMENT

Except in the starch-and sucrose-based beverage industries, most research on the fermentative conversion of carbohydrates to ethanol and other neutral products ended in the early 1950's with the advent of cheap petroleum and natural gas. Since that time, however, knowledge of microbial biochemistry and genetics has grown enormously.

The current and projected scarcity of liquid and gaseous fossil fuels has stimulated interest in the production of substitutes derived from renewable (biomass) sources. To some extent, traditional starch-and sucrose-based fermentations can be expanded to increase supplies of liquids fuels, but since these substrates are also used for human and animal food, their usefulness as fuel sources is limited. Sugars derived from the cellulose and hemicellulose components of biomass are more attractive as fuel sources since in many cases they are not presently utilized or have a lower value than the potentially derivable fuels.

With respect to the pentose fraction of biomass, the microorganisms described in this review all have the ability to produce ethanol and/or other neutral products from this source, and it is possible that in the near future the older technologies described might become economic. However, application of modern knowledge could yield even greater economies.

Fusarium oxysporum (lini) appears to have the ability to produce almost stoichiometric conversions of pentose to ethanol and carbon dioxide, but the growth and fermentation rates associated with the organism make its use currently uneconomic (18). Altered nutritional or environmental parameters might allow faster growth as might the selection of genetically modified "fitter" (65) mutants.

As mentioned in the previous discussion, extracts of some strains of brewers yeast appear to be able to ferment pentose to ethanol and  $\text{CO}_2$ , but all the substrate carbon was not accounted for. It was suggested that the lost carbon was present in a pool of metabolic intermediates. This should be verified. Studies of the regulation of the pentose and glycolytic enzymes involved should suggest approaches to be used for the selection of pentose-fermenting mutants.

Organisms which conduct mixed acid and modified mixed acid fermentations of pentose (and hexose) sugars yield organic acids as well as neutral products. These acids are usually not desirable end products. Knowledge of the regulation of these pathways should suggest biochemical strategies for minimizing acid production in fermentations. Metabolically blocked mutants could also be employed.

All of the above approaches might be utilized in the butanediol and butanol fermentations, and in addition improved methods for separating these high-boiling products should be sought.

Finally, consideration should be given to the idea that the organisms that have been described in the past are not necessarily the only ones of interest. New organisms displaying desirable new fermentation patterns remain to be discovered.

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Table 1. Percent composition of dry agricultural residues and woods. (5).

<u>MATERIAL</u>	<u>HEXOSANS</u>	<u>PENTOSANS</u> <sup>a</sup>	<u>LIGNIN</u>	<u>ASH</u>
Barley Straw	40	19	14	11
Corn Stover	36	16	15	4
Cotton Gin Trash	20	6	18	15
Rice Hulls	36	15	19	20
Rice Straw	39	17	10	12
Sorghum Straw	33	18	15	10
Wheat Straw	36	19	15	10
Corn Stalks	35	15	19	5
Sycamor	46	14	22	1
Sweet Gum	45	19	19	1
White Fir	58	9	28	N.D.
Douglas Fir	57	4	27	0.2
Redwood	44	6	34	0.4

a) Polymerized xylose and arabinose

N.D.—not determined

Table 2. Product yields during fermentation of D-xylose by growing cultures of F. oxysporum (94% of substrate carbon accounted for)(18).

PRODUCT	gm/gm XYLOSE FERMENTED	MOLE/100 MOLES XYLOSE FERMENTED
Cell Mass	0.12	----
Ethanol	0.41	134
Carbon Dioxide	0.32	109
Acetic Acid	0.04	10

Table 3. Fermentative patterns of members of the enteric group using glucose as a substrate <sup>a</sup>(22)

FERMENTATION TYPE			
GENUS	MIXED ACID	MIXED ACID + 2,3 BUTANEDIOL	PRESENCE OF FORMIC HYDROGENLYASE
ESCHERICHIA	✓		✓
SALMONELLA	✓		✓
SHIGELLA	✓		
ENTEROBACTER		✓	✓
SERRETIA		✓	
PROTEUS	✓		✓
ERWINIA		✓	
YERSINIA	✓		
VIBRIO	✓		
AEROMONAS	(✓)	(✓)	(✓)
BENECKEA	(✓)	(✓)	
PHOTOBACTERIUM	(✓)	(✓)	(✓)

(a) ✓ = most species

(✓) = some species

Table 4. Millimoles of products formed per 100 mM of substrate fermented by E. coli strains.

<u>PRODUCT</u>	STRAIN PRLR <sub>2</sub> -GLUCOSE (35)		STRAIN ATCC 26 <sup>a</sup> (36)	
	<u>PH 6.0</u>	<u>pH 7.0</u>	<u>GLUCOSE</u>	<u>XYLOSE</u>
2,3 BUTANEDIOL	0.30	0.26	--	--
ACETOIN	0.06	0.19	--	--
ETHANOL	49.8	50.5	37.0	17.7
GLYCEROL	1.42	0.32	--	--
BUTYRIC ACID	--	7.10	--	--
ACETIC ACID	36.5	38.7	30.2	17.6
FORMIC ACID	2.43	86.0	5.8	3.0
SUCCINIC ACID	10.7	14.8	4.9	29.1
LACTIC ACID	79.5	70.0	119.2	106.2
CARBON DIOXIDE	88.0	1.75	56.4	4.5
HYDROGEN	75.0	0.26	53.7	3.6
% CARBON RECOVERED	91.2	94.7	95.5	103
O/R INDEX (43)	1.06	0.91	1.03	1.05

a) Final pH controlled to 5.6-5.8 with CaCO<sub>3</sub>.

Table 5. Fermentation products of Ruminococcus albus, strain 56-2, (25) and Clostridium thermocellum, strain 651, (27) grown with cellulose as a carbon source.

PRODUCT	mM/100 mM HEXOSE FERMENTED	
	<u>R. ALBUS</u>	<u>C. THERMOCELLUM</u>
H <sub>2</sub>	41.7	44
CO <sub>2</sub>	19.8	56
ETHANOL	55.9	35
ACETIC ACID	81.0	17
FORMIC ACID	---	4
LACTIC ACID	102.2	21
SUCCINIC ACID	---	---
% CARBON RECOVERY	100	102

Table 6. Mixed Acid, 2,3-butanediol fermentation of glucose and xylose. mm product/100 mm sugar fermented.

PRODUCT	AEROMONAS HYDROPHILA (37)		BACILLUS POLYMYXA (38)		AEROBACTER INDOLI GENES (36)		AEROBACTER (ENTEROBACTER) AEROGENES (31)	
	GLUCOSE	XYLOSE	GLUCOSE	XYLOSE	GLUCOSE	XYLOSE	GLUCOSE, PH 5.2	GLUCOSE PH 7.6
2,3-BUTANEDIOL	54.7	39.0	65.1	38.0	64.1	44	48.8	--
ACETON	1.7	2.6	2.8	2.5	0.7	0.92	1.39	3.85
ETHANOL	52.0	48.9	66.2	63.0	66.6	55.9	61.2	54.7
GLYCEROL	--	--	--	--	--	--	1.81	7.72
ACETONE	--	--	--	--	--	--	0.53	--
BUTYRIC ACID	--	--	--	--	--	--	0.29	1.79
ACETIC ACID	4.6	9.3	2.9	7.7	1.0	11.4	3.89	52.7
FORMIC ACID	--	--	--	--	27.9	26.3	0.43	52.4
SUCCINIC ACID	3.6	1.1	--	--	--	5.5	2.17	4.10
LACTIC ACID	23.3	20.4	--	--	3.0	5.2	2.34	36.5
CARBON DIOXIDE	166.2	134.7	199.6	161.0	153	114	183.8	77.4
HYDROGEN	57.5	53.9	70.9	82.0	27.6	19.1	69.8	69.7
% CARBON RECOVERED	98.2	96.6	101.6	92.9	97.0	98.5	97.0	94.9

Table 7. Acetone-Butanol-Ethanol Fermentations of Glucose.  
mM of product per 100 mM Glucose Fermented. ( 43 ).

PRODUCT	ORGANISM		
	<u>CLOSTRIDIUM PERFRINGENS</u>	<u>CLOSTRIDIUM ACETOBUTYLICUM</u>	<u>CLOSTRIDIUM BUTYLICUM</u>
BUTYRIC ACID	34	4.3	17.2
ACETIC ACID	60	14.2	17.2
LACTIC ACID	33	--	--
CARBON DIOXIDE	176	221	203.5
HYDROGEN	214	135	77.6
ETHANOL	26	7.2	--
BUTANOL	--	56	58.6
ACETONE	--	22.4	--
ACETOIN	--	6.4	--
ISOPROPANOL	--	--	12.1



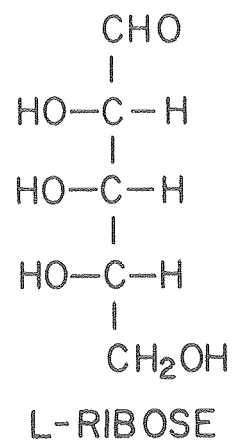
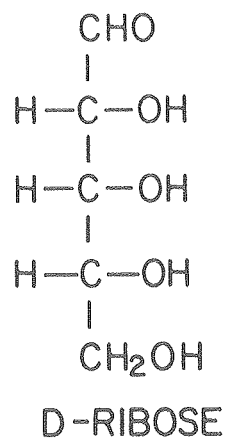
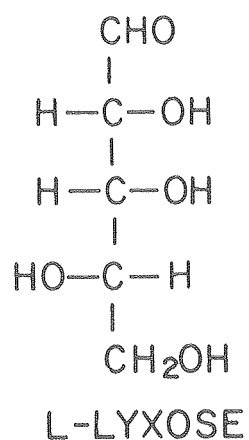
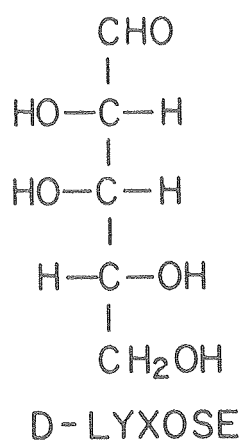
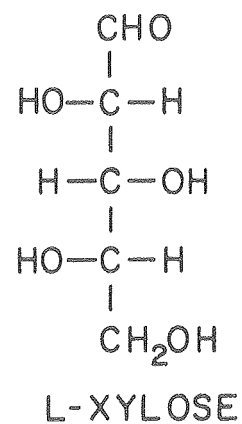
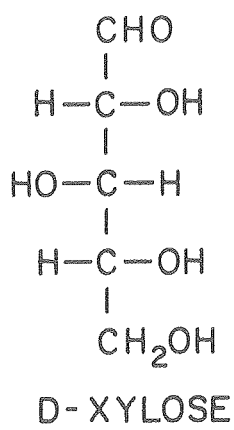
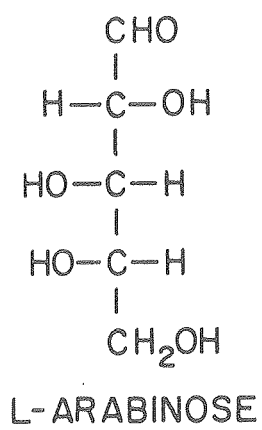
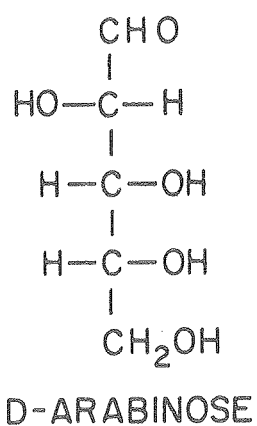
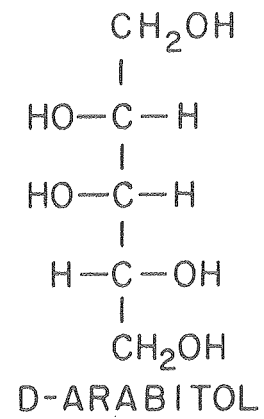
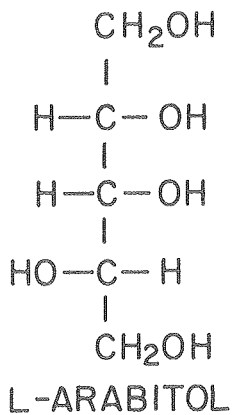
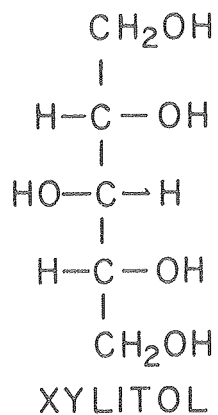
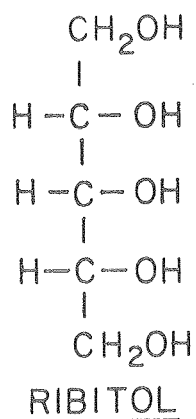
Table 8. Yield of neutral products formed by Bacillus macerans growing with various carbon sources.  
 Medium: 2% sugar, 0.5% peptone, 2%  $\text{CaCO}_3$  (55).

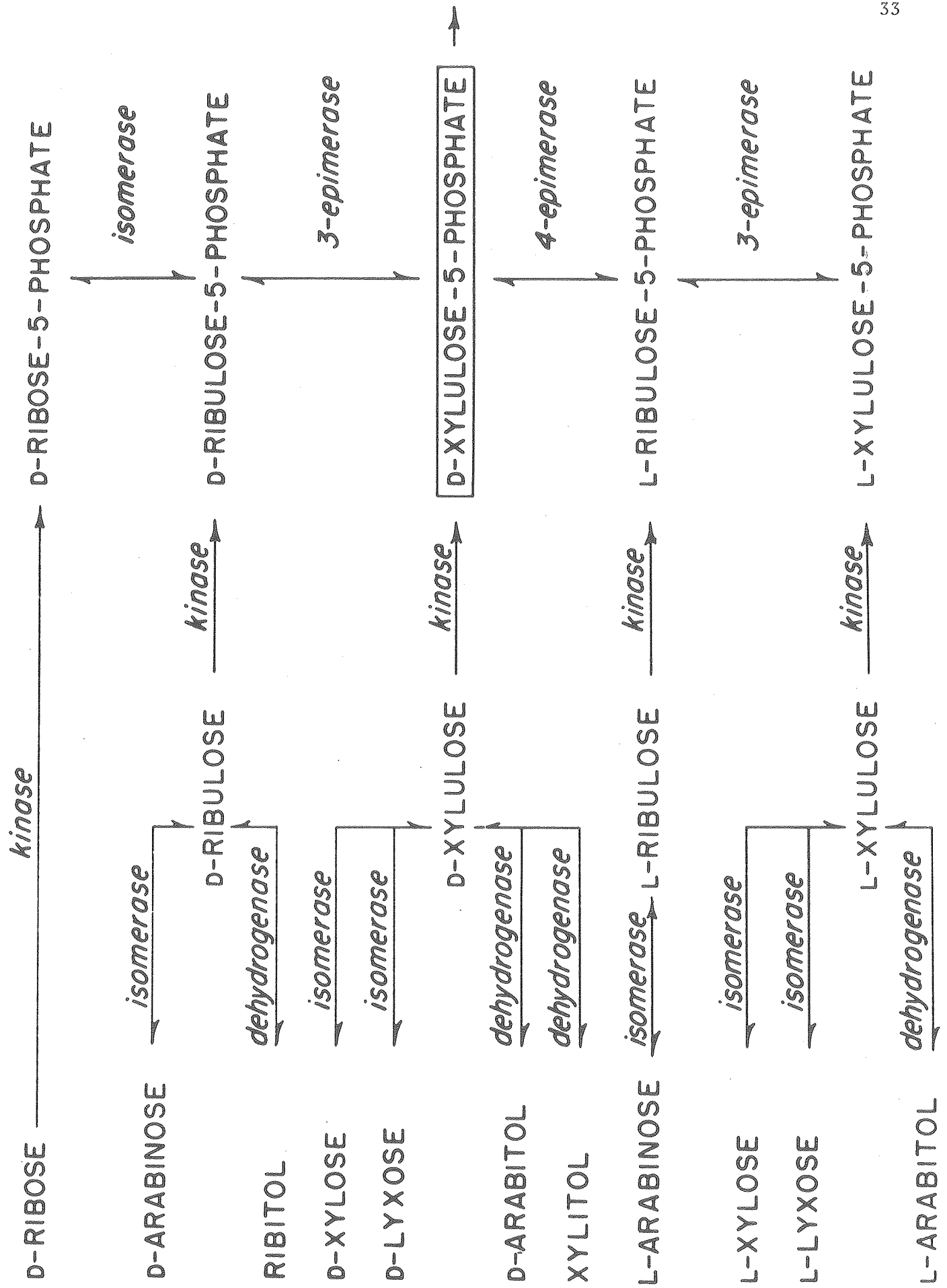
<u>SUBSTRATE</u>	<u>g PRODUCT/100 g SUBSTRATE USED</u>	
	<u>ACETONE</u>	<u>ETHANOL</u>
GALACTOSE	4-5	22-24
MALTOSE	6-7	23-24
MANNOSE	6-7	22-23
RAFFINOSE	8-10	22-23
L-ARABINOSE	6-7	12-16
STARCH	8-10	20-24
DEXTRIN	6-7	14-16
GLUCOSE	9-10	22-23
FRUCTOSE	8-10	24-25
XYLOSE	4-5	18-20
GLYCEROL	---	40-43
SUCROSE	8-9	24-26

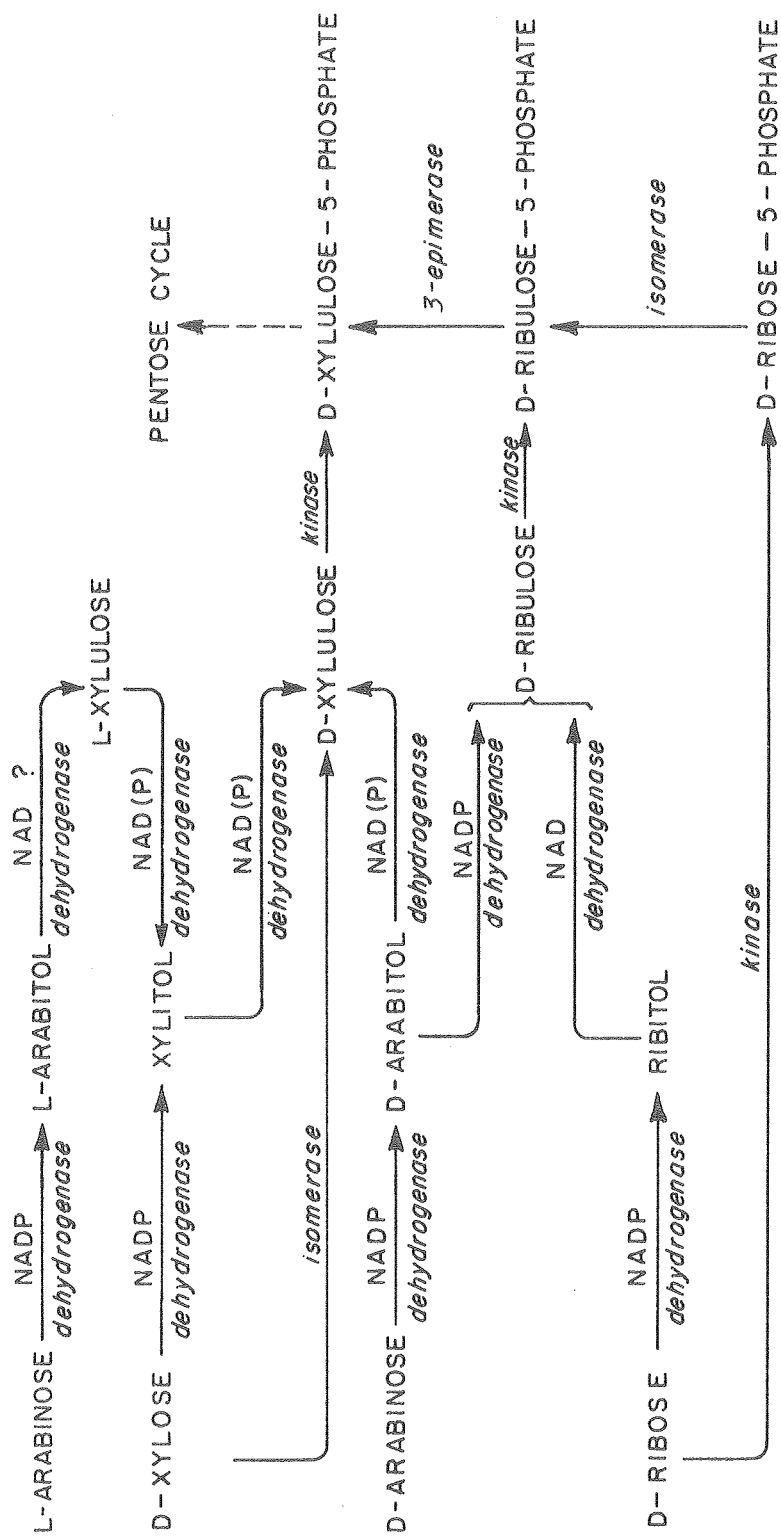
FIGURES

- FIGURE 1                    Chemical structures of pentitols and pentoses.  
Naturally abundant species are underlined.
- FIGURE 2                    Pathways of pentose and pentitol metabolism in  
Klebsiella pneumoniae, PRL-R3 (8).
- FIGURE 3                    Pathways of pentose and pentitol metabolism in yeast (12).  
Abbreviations used in this and following figures:  
P, phosphate; NAD, nicotinamide adenine dinucleotide;  
ATP, adenosine triphosphate; ADP, adenosine diphosphate;  
CoA, Coenzyme A; Fd, ferredoxin.
- FIGURE 4                    Pathway for the conversion of pent(ul)ose phosphate to  
pyruvic acid in pentose-fermenting bacteria.
- FIGURE 5                    Pathway for the conversion of xylulose-5-phosphate to  
acetic acid and pyruvic acid in resting cells of F.  
oxysporum f. sp. lini (Bolley).
- FIGURE 6                    Steps in the conversion of pyruvic acid to ethanol and  
CO<sub>2</sub> used by F. oxysporum and yeast.

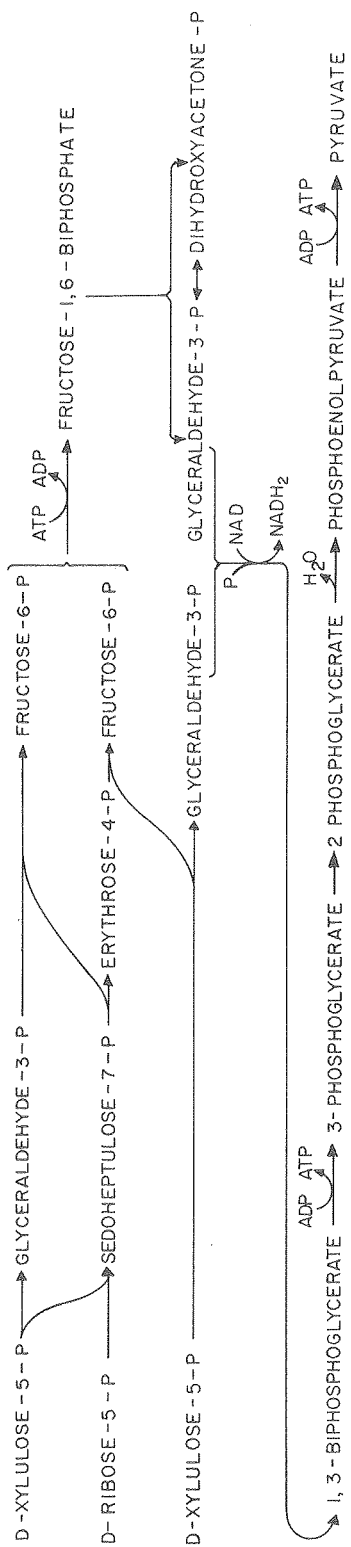
- FIGURE 7. Pathways for the formation of products of the mixed acid and 2,3-butanediol fermentations.
- FIGURE 8 Pathways for the formation of acetone, butanol, butyric acid and isopropanol from pyruvate.
- FIGURE 9 Effect of pH on product yields from Bacillus macerans fermentation (56).



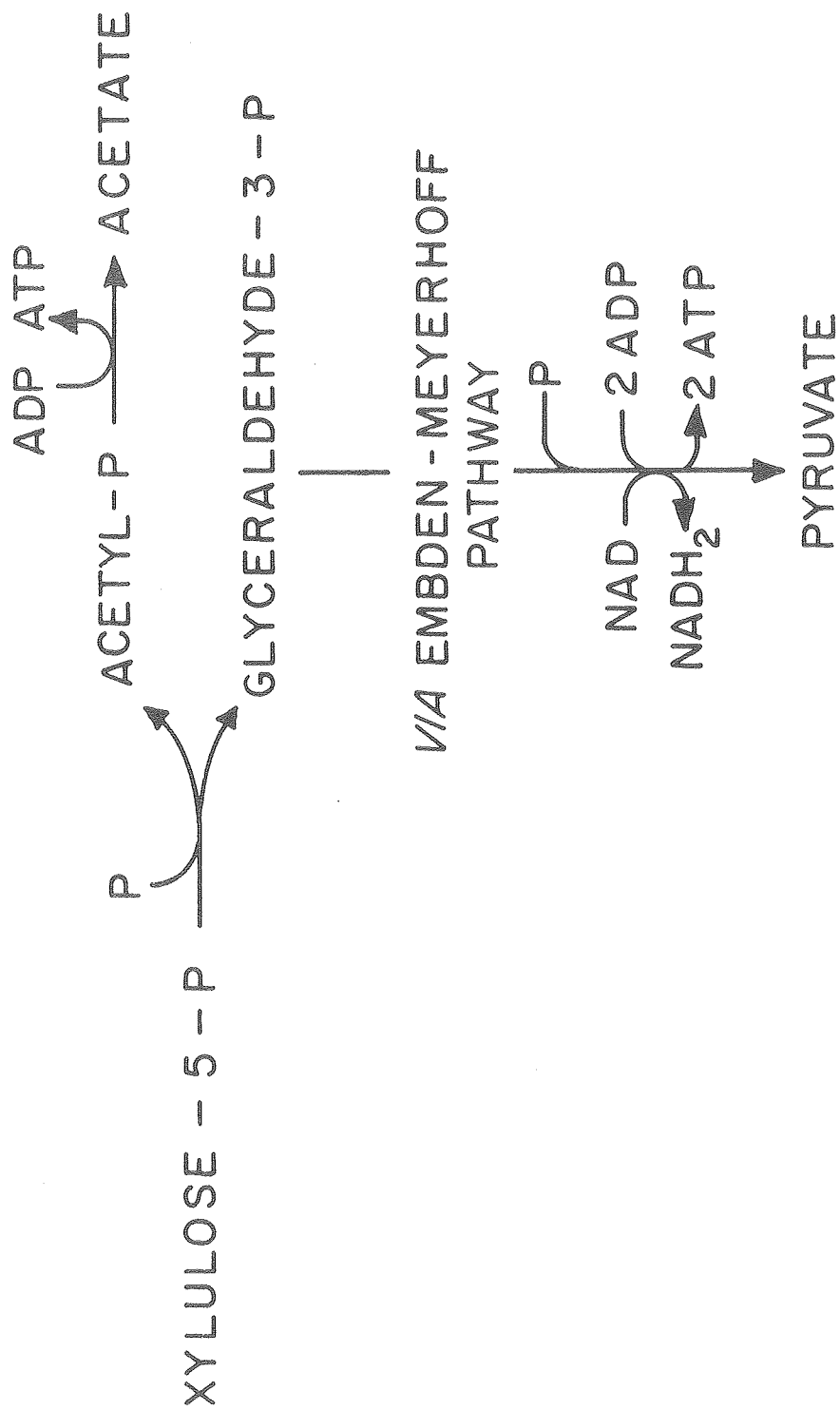




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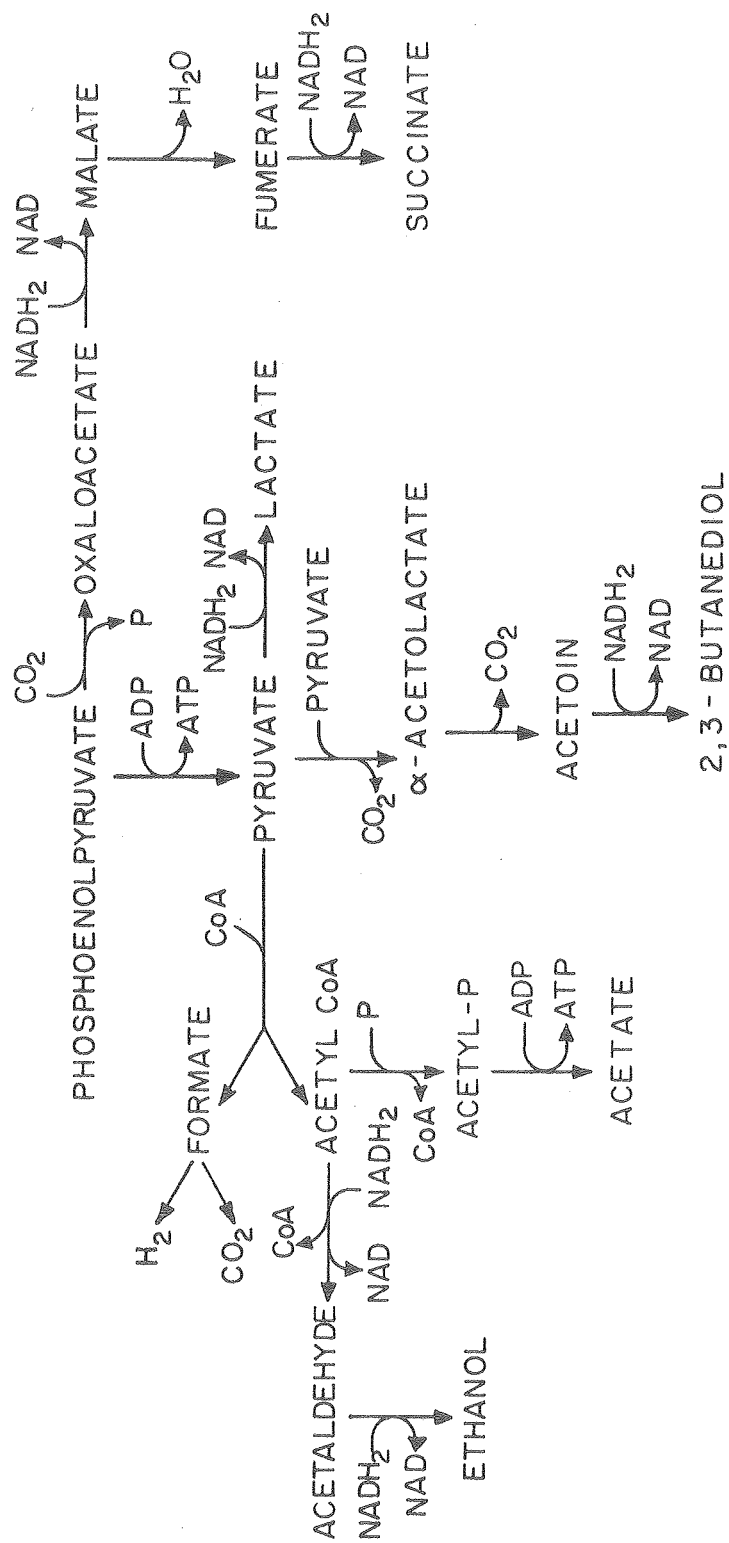


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